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Population ecology and habitat preferences of juvenile flounder *Platichthys flesus* (Actinopterygii: Pleuronectidae) in a temperate estuary

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Abstract

The European flounder *Platichthys flesus* is a widely distributed epibenthic species and an important component of demersal fish assemblages in the European Atlantic coastal waters. In Portuguese estuaries, this species reaches high densities, especially in Minho estuary (NW Iberian Peninsula, Europe), potentially playing an important role in the system's ecology. In this context, the population structure, production and the habitat use of juvenile *P. flesus* were investigated. Sampling took place monthly, from February 2009 until July 2010 along the entire estuarine gradient (5 sampling stations distributed in the first 29 km from the river mouth, with S1 located near the river mouth, S2 inside

a salt marsh, S3 in a salinity transition zone, while S4 and S5 were located in the upper estuary). Flounder's density varied significantly among sampling stations and seasons (Two-way PERMANOVA: $p < 0.001$), with the majority of the individuals being found during the spring (30.1%) and in S3 and S4 (72.6%). Males and females presented an even distribution, with a higher proportion of males observed during summer. Fish length also differed among sampling stations and seasons (Two-way PERMANOVA: $p < 0.001$), with larger fishes being found in S1 during the autumn (168.50 ± 59.50 mm) and the smallest in S4 during the spring (33.80 ± 3.12 mm). Size classes associated differently with environmental variables, with larger juveniles being more abundant in the downstream areas of the estuary, whereas smaller juveniles were related to higher water temperatures, suggesting a habitat segregation of *P. flesus* of different sizes. The fish condition of *P. flesus* in Minho estuary was higher than in other systems, probably due to the dominance of juveniles on the population. Also, the densities found in this estuary were up to 32 times higher than in other locations, suggesting that Minho estuary is an important nursery area for the species. The estimated secondary production of *P. flesus* was lower than previous studies acknowledged in the system ($0.037 \text{ g.WWm}^{-2}.\text{year}^{-1}$), indicating that the production of this species in estuaries can vary considerably depending of several factors such as the sampling year and strategy, population and fish size.

Key-words: flatfish / migration / nursery ground / secondary production / Portugal

1. Introduction

The spatial distribution of organisms is generally not homogeneous in space and time as a result of habitat heterogeneity and complex mechanisms (Chesson, 1998).

Environmental-driven habitat selection is directly related to individual-level responses to different biotic and abiotic features that unequally are distributed in space across habitats. This results in how different proportions of a population use different habitats (Křivan and Sirot, 2002). Habitat features such as depth, current, substratum type (e.g. Schlosser, 1982; Bain et al., 1988; Ramos et al., 2009), presence of shelter or habitat diversity (Gorman and Karr, 1978; M  rigoux et al., 1998) may play a major role in shaping fish population in space; whereas precipitation, river discharge, water temperature and salinity (e.g. Matthews, 1998; Martinho et al., 2009) are the main physical parameters structuring fish population in time.

In estuaries, the habitat heterogeneity is particularly high, with marked dissimilarities in space and time of the abiotic parameters such as salinity, temperature, flow and tidal energy; and therefore for the associated flora and fauna (Hoffman et al., 2008). Additionally, estuaries contain diverse aquatic habitat types, including seagrass, kelp and shellfish beds, hard-bottom, soft bottom communities with mud and sand, rocky inter-tidal zones, fringing mangrove forests and vegetated marshes/wetlands (Nelson and Monaco, 2000). Thus, the heterogeneity of this ecosystem is ideal for developing generalities about population ecology and habitat preferences, especially for those euryhaline species which can inhabit the saline, brackish and the freshwater portions of the estuary.

Estuaries are also highly productive environments that provide important forage, spawning, refuge, and nursery habitat for commercial, recreational, and forage fish species during one or more of their life history stages (Chambers 1992; Nelson and Monaco 2000; Able, 2005). Thus, estuaries are considered an essential habitat for many fish species at various stages in their development (Able, 2005).

Interestingly, several fish species also change their habitat preferences during growth, with habitat relationships associated with particular life history stages reflecting the changes in morphology, physiology and trophic guild membership that occur throughout ontogeny (Swain et al., 1998; Garrison and Link, 2000).

Flatfishes of the Pleuronectidae family are a worldwide spread group of fishes, occurring in fresh, brackish and marine waters. Pleuronectidae includes 104 species distributed in 41 genera (Froese and Pauly, 2012), and it is among the most ecological relevant fish family in European estuaries. Therefore, its ecology has received much attention in several estuarine systems across the continent (e.g. Becker, 1988; Van der Veer et al., 1991; Modin and Pihl, 1996; Cabral et al., 2007; Ramos et al., 2009, 2010).

One of the most important flatfish species from European waters is the European flounder *Platichthys flesus*. This flounder is a widely distributed species in coastal and brackish waters, naturally occurring in the Black Sea, the Mediterranean Sea, the European Atlantic Coast (including the British Isles and Ireland), the North, the Baltic, the Barents and the White Sea (Nielsen, 1986; Rochard and Elie, 1994). The described distribution of *P. flesus* in the Atlantic waters ranges from Norway to Morocco (Nielsen, 1986), but more recently, other studies pointed out that the northern and central coastal areas of Portugal are more likely to be the current southern limit for its distribution (Cabral et al., 2007; Dolbeth et al., 2008). Some of these estuaries were already identified as important nursery grounds for flounder, particularly Mondego, Ria de Aveiro, Douro, Lima and Minho (Cabral et al., 2007; Vasconcelos et al., 2008, 2010; Freitas et al., 2009; Ramos et al., 2010). From these, Minho estuary has the highest density values, $2.93 \text{ ind.}100\text{m}^{-2}$ (Freitas et al., 2009), which are up to 11 times higher than the maximum values found in other Portuguese estuaries (e.g. Vinagre et al., 2005;

Pombo et al., 2007; Martinho et al., 2007; Ramos et al., 2010; Vasconcelos et al., 2010; França et al., 2011).

Freitas et al. (2009) found that *P. flesus* population in Minho estuary is largely dominated by juveniles of 0- and 1-year group individuals, with upstream zones (freshwater) of the estuary being the preferred by the species. Moreover, in a recent study on flounder migration patterns, it is discussed the possibility of Minho estuary to be also a spawning area for this species, rather than just a nursery and feeding ground, as previously described (Morais et al., 2011). The European flounder have been receiving an increasing attention by scientists in the past years (e.g. Freitas et al., 2009; Morais et al., 2011). Nevertheless, there are still lacks of information in the patterns of population function and structure in Minho estuary, particularly related to fish-habitat association, recruitment and secondary production. Due to the economic and ecological relevance of *P. flesus*, the continuous monitoring of the different populations across the entire range of distribution is recommended by the IUCN to determine stock status of the species (Munroe, 2010). In this context, the present field-based study aimed to assess the habitat use of the European flounder in an estuary where the species reaches remarkable high values of density (Freitas et al., 2009). Our goals were twofold: (a) to investigate the population structure, the recruitment pattern, the secondary production and the distributional patterns of *P. flesus* juveniles along the entire salinity gradient (saline, brackish and freshwater) and (b) to investigate the spatio-temporal habitat preferences of the European flounder juveniles of different sizes. By focusing on the distributional patterns of *P. flesus* within a system where the species seems to be especially abundant, we expect to increase the understanding on the ecology of a key-species in European estuaries.

2. Material and Methods

2.1. Study area and sampling campaign

The Minho estuary is located in the NW-Iberian Peninsula (SW Europe) and covers a total area of 23 km². The limit of tidal influence is about 40 km inland, and the uppermost 30 km are a tidal freshwater wetland. The estuary is mesotidal, with tides ranging between 0.7 m and 3.7 m (Alves, 1996). The mean depth of the estuary is 2.6 m and the maximum depth is about 26 m (Antunes et al., 2011), and the average annual freshwater run-off is about 300 m³.s⁻¹ (Ferreira et al. 2003).

The present study was carried out in five stations along the entire salinity gradient. Sampling was conducted during 18 consecutive months, from February/09 to July/10 in four subtidal areas (S1, S2, S3 and S4) and during 12 consecutive months (from February/09 to January/10) in the most upstream sampling station (S5) (Fig. 1). The five stations were selected in order to assess possible dissimilarities on the population structure related to differences in the habitat type and environmental characteristics.

S1 is located near the river mouth (*ca.* 1.5km), and characterized by muddy soft bottoms often densely covered by debris, such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al., 2011). S2 is located inside the Coura salt marsh (*ca.* 3.5km apart from the river Minho mouth), which is a relatively small sub-system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channels' soft bottom is sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al., 2011). S3 is located *ca.* 8 km upstream from the river mouth and characterized by presenting high densities of the Asian clam *Corbicula fluminea* and by the soft bottom sparsely covered by debris and submerged vegetation (Sousa et al., 2008a, 2008b; Ilarri et al., 2012). S4 is located in the freshwater portion of the estuary (*c.a.* 21 km upstream from the river mouth) and

characterized by presenting high densities of submerged vegetation, specially water crowfoots (*Ranunculus* sp.) and high densities of the Asian clam *C. fluminea*. S5 is located *c.a.* 29 km upstream from the river mouth and *c.a.* 1km upstream from the Louro river (tributary to the Minho river). This station is characterized by presenting freshwater (salinity < 0.05) during almost the entire year, the bottom is also covered by Asian clams and submerged vegetation.

In each station, three replicates per month were collected during the day at high tide of spring tides using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h⁻¹). Duration of the tows varied between 2 to 3 minutes depending on the sediment type, in order to avoid net clogging by detritus or obstruction of the gear. The sampling area covered by a 3 min tow corresponds to 100 ± 4 meters (Freitas et al., 2009). Additionally, environmental variables (water temperature, salinity, pH and oxidation reduction potential (ORP)) were measured with a multiparameter probe YSI 6820 deployed to 20 cm off the bottom.

The monthly river discharge data measured at Foz do Mouro hydrometric station between February 2009 and July 2010 was obtained from the INAG – Instituto da Água, I.P. (<http://snirh.inag.pt>).

2.2. Laboratory procedures

The density of *P. flesus* juveniles was determined by counting all individuals caught in each sampling station. Size (standard length – *SL* and total length – *TL*) of individual fish was measured (0.5mm precision) using a ruler and subsequently, all individuals were assigned into six different size classes based on *SL*: F1 (< 25.0 mm), F2 (25.0 to 49.9 mm), F3 (50.0 to 74.9 mm), F4 (75.0 to 99.9 mm); F5 (100.0 to 125.0 mm) and F6

(> 125.0 mm). Individuals larger than 185 mm of TL were considered adults (Kosior et al., 1996).

All individuals had their sex identified based upon dimorphic features on their morphology and gonads, in order to compare the spatio-temporal distribution and habitat use of juveniles of different sexes. Additionally, all *P. flesus* individuals were wet weighted in order to determine the total biomass captured in Minho estuary.

The condition factor of *P. flesus* was calculated using the allometric equation:

$$(1) \quad W = a \cdot L^b$$

Where W is the wet weight of the fish (in grams), L is the total length of the fish (in centimeters), a is the coefficient related to body form and b is an exponent indicating isometric growth when equals to 3 (Reiss, 1989; Beverton and Holt, 1996).

2.3. Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated.

The Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (5 levels: S1, S2, S3, S4 and S5) and season as a fixed factor (4 levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season and in each station), and calculates a pseudo-*F* (based

on permutations) which is identical to the F statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson, 2001).

The Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data.

Similarly, the flounder data were also normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each sex (male and female), the density of the six size classes and the size of males and females *P. flesus* individuals were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (5 levels: S1, S2, S3, S4 and S5) and season as a fixed factor (4 levels: winter, spring, summer and autumn), using all both multivariate and univariate approaches (comparing each variable separately).

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

The PCA and PERMANOVA analyses were performed using PRIMER v 6.1.11[®] (Clarke and Gorley, 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al., 2008).

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of each size class of *P. flesus* and abiotic data using CANOCO 5 software (ter Braak and Verdonschot, 1995). The Monte-Carlo

randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (see ter Braak, 1986).

The population structure of *P. flesus* was defined by tracking recognizable cohorts from the successive sampling dates. Spatial samples were pooled together and analyzed using the size frequency distribution of successive dates. The mixture analysis method was used to identify cohorts within size frequency distributions constructed from population samples in each month using the PAST[®] software. The mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion), of univariate normal distributions, based on a pooled univariate sample. This software uses the EM algorithm described by Dempster et al. (1977) and considers a histogram of frequency as a mixture of probability density functions. The number of modes in each month was determined by visual analysis of the histogram.

The secondary production was computed using the size-frequency method (Hynes method), which calculates production by summing the biomass lost between size classes (Krueger and Martin, 1980). The annual production of *P. flesus* in Minho estuary was calculated according to the Krueger and Martin (1980) equation:

$$(2) \quad P = \sum_{j=1}^{a-1} 0.5 [(Y_{i,j} + Y_{i+1,j}) - (Y_{i,j+1} + Y_{i+1,j+1})] \cdot (W_j \cdot W_{j+1}) \cdot (t)^{-1}$$

where P is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m⁻²) of the size class j in the date i , $Y_{i+1,j}$ is the mean density of the size class j in the date $i+1$, $Y_{i,j+1}$ is the mean density of the size class $j+1$ in the date i , $Y_{i+1,j+1}$ is the mean density of the size class $j+1$ in the date $i+1$, W_j is the biomass (wet weight) (g.m⁻²) of the size class j , W_{j+1} is the biomass (wet weight) of the size class $j+1$ and t is the time between i and $i+1$. Population production estimates correspond to the sum of each size class

production (P). Negative production values were not included in the overall estimates and were regarded as zero production.

3. Results

3.1. Abiotic data

The two-way PERMANOVA for stations and seasons of the abiotic variables differences was highly significant for both main effects (stations: pseudo- $F = 8.30$, $p < 0.001$; seasons: pseudo- $F = 9.12$, $p < 0.001$) but not for the *stations x seasons* interaction (pseudo- $F = 1.01$, $p = 0.45$). When analyzed independently, each abiotic variable displayed a different pattern. The variables that varied the most among sampling stations and seasons were depth, salinity and temperature, while pH varied slightly, and ORP was not different among sampling stations and seasons. Overall, the pairwise tests of the two-way PERMANOVA indicated that the depth was lower in S2 and S3 compared with other stations. Salinity varied among sampling stations, with S1 presenting higher values than other stations; the only station where significant differences in salinity were detected among seasons was S3, which presented significantly saltier waters during summer. Temperature varied seasonally, with winter differing from other seasons; moreover, water temperature was significantly higher in S4 and S5 during summer compared to other stations (Table 1).

The PCA routine indicated that the first two axis of the PCA explained 55.8% of the variance among samples and revealed differences between sampling stations, with S1 and S2 clearly differing from S4 and S5, while S3 was associated to both main groups (Fig. 2). The pH (eigenvalue = -0.628) and the water temperature (eigenvalue = -0.566) presented the strongest correlations with PC1, while depth (eigenvalue = 0.749) was the most important variable in explaining PC2 (Fig 2).

3.2. Flounder data

A total of 528 fishes were collected ($S3 = 43.6\%$, $S4 = 29.0\%$, $S2 = 11.9\%$, $S5 = 9.3\%$ and $S1 = 6.2\%$). Of these, 30.1% were sampled during spring, 24.8% in summer, 24.5% in autumn and 20.6% during winter. Males and females were evenly found in Minho estuary (46.6% and 46.8% of the total respectively), while undefined individuals accounted only for 6.6% of the total number of individuals sampled.

3.2.1. Density patterns

The mean density of *P. flesus* in Minho estuary, considering the entire sampling period and the 5 stations, was $2.84 \pm 0.27 \text{ ind.100m}^{-2}$ (mean \pm SE). The density of *P. flesus* varied throughout the sampling period, with higher values being found during the spring ($4.47 \pm 2.31 \text{ ind.100m}^{-2}$ in May/09) and summer months ($4.60 \pm 1.24 \text{ ind.100m}^{-2}$ in August/09) (Fig. 3A).

The two-way PERMANOVA for stations and seasons of *P. flesus* density differences was significant for the *stations x seasons* interaction (pseudo- $F = 2.28$, $p < 0.05$). The density of *P. flesus* was even throughout seasons in S1, S3 and S5, while in S2, a significant higher number of individuals were captured during the summer ($2.75 \pm 0.96 \text{ ind.100m}^{-2}$) in comparison with autumn ($0.44 \pm 0.29 \text{ ind.100m}^{-2}$) (Two-way PERMANOVA, pairwise test: $t = 2.33$, $p < 0.05$), and in S4 the density was higher during the autumn ($8.44 \pm 1.45 \text{ ind.100m}^{-2}$) in comparison with the other three seasons (Two-way PERMANOVA, pairwise tests – *Winter x Autumn*: $t = 4.53$, $p < 0.001$; *Spring x Autumn*: $t = 2.12$, $p < 0.05$; *Summer x Autumn*: $t = 2.37$, $p < 0.05$). Overall, the density of *P. flesus* was higher in S3 during all seasons, except during autumn, when S4 presented the highest density (Fig. 3B).

During winter, spring and summer, most of the *P. flesus* individuals (71.4%, 70.3% and 58.3% respectively) were captured in the three stations located in the lower and in the intermediary estuary (S1, S2 and S3); while during the autumn, the pattern was different with 69.0% of the individuals being caught in the sampling stations located in the upper estuary (S4 and S5).

Overall, males and females presented an even distribution along the five sampling stations and the four seasons, with a higher sex ratio (Male/Female) being found during the summer (1.30), followed by winter (0.99), spring (0.81) and autumn (0.70) respectively. The density of males was slightly higher than females' in stations S1 and S3, whereas the females' density was slightly higher in stations S2, S4 and S5. Additionally, the interaction effect of stations and seasons for the density males was significant, with higher density values being recorded in S3 and S4 during spring and autumn respectively. Females also showed a similar pattern, with higher densities being recorded in S4 and S3 during autumn and spring respectively (Table 2).

3.2.2. Size and recruitment patterns

Almost the entire population was composed by juveniles (only one adult individual was captured in S1), and the range of *SL* varied between 7.5 and 184.0 mm, with an average value of 54.90 ± 1.38 mm (mean \pm SE). The two-way PERMANOVA for stations and seasons of *P. flesus* size differences was significant for both main effects (stations: pseudo- $F = 54.22$, $p < 0.001$; seasons: pseudo- $F = 22.75$, $p < 0.001$), and for the *stations x seasons* interaction (pseudo- $F = 2.63$, $p < 0.001$).

When analyzing fish size of each sex separately, both males and females were frequently larger in S1 and smaller in the stations located in the intermediate (S3) and

the upper zone (S4) of the estuary. Additionally, *P. flesus* individuals were often smaller during the spring and larger during the autumn (Table 3).

The two-way PERMANOVA for stations and seasons of the European flounder size classes densities was significant for both main effects (stations: pseudo- $F = 5.11$, $p < 0.001$; seasons: pseudo- $F = 2.21$, $p < 0.01$) and for the *stations* \times *seasons* interaction (pseudo- $F = 1.81$, $p < 0.001$).

Overall the Minho estuary population is dominated by small-bodied individuals, with the smaller size classes (F1, F2 and F3) representing at least 52% of the population throughout the entire study period, and reaching up to 94% of the population in April/09 (Fig. 4A).

The mixture analysis indicated that the recruitment of *P. flesus* occurred once a year (March/09 and May/10) in in Minho estuary (Fig. 4B).

3.2.3. Fish-habitat associations

Overall, size classes associated differentially with environmental variables. The smallest fishes (F1) tended to be associated with high water temperature and low river discharge and salinity, whereas larger fishes (F5 and F6) were associated with high values of salinity and depth. On the other hand, intermediate size fishes (F2, F3 and F4) showed weaker associations with environmental variables (Fig. 5). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.39; F-ratio = 2.92; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 86.3% of the total variance, with correlations between *P. flesus* size classes densities and environmental variables of 0.574 (axis 1) and 0.536 (axis 2).

No significant associations were found between the density of males and females and the environmental variables (test of significance of all canonical axes: trace = 0.10; F-

ratio = 0.81; $p = 0.56$), indicating that males and females have similar habitat preferences in Minho estuary.

3.2.4. Length-weight relationship and secondary production

During the study period, a total of 2739 g (wet weight) of *P. flesus* was collected in Minho estuary. Males presented a ratio of TL/wet weight slightly higher than females. When comparing the results of the length and weight relationship of the *P. flesus*, the values found in Minho estuary was different from others, since the allometric coefficient a values were lower and the allometric coefficient b values were higher than other locations (Table 4).

The overall the secondary production of *P. flesus* in Minho estuary was of 0.052 g.WWm^{-2} , with the annual production of $0.037 \text{ g.WWm}^{-2}.\text{year}^{-1}$.

4. Discussion

4.1. Distribution patterns and habitat use

European flounder juveniles presented spatial and temporal differences in its distribution in Minho estuary. Spatially, *P. flesus* seemed to prefer the intermediate (S3) and upper (S4) zones of the estuary. Interestingly, these two stations are about 14 km apart from each other and present marked differences in their environmental characteristics, mainly related to depth and salinity. While in S3 the salinity values varied between 0.03 (February/09) and 27 (August/09), the S4 presented salinity values constantly lower than 0.05; additionally, S4 ($4.1 \pm 1.38 \text{ m}$) is much deeper than S3 ($2.3 \pm 0.87 \text{ m}$) and presented important differences in the benthic habitat characteristics, once in S4, the submerged vegetation is copious, whereas in S3 the presence of the Asian clam *C. fluminea* is high. Despite the abovementioned differences in habitat

characteristics, both sampling stations sustained high densities of *P. flesus* throughout the whole study period, indicating that other factors not measured in this study may be also important to *P. flesus*. The European flounder is able to tolerate a wide range of salinity (Nielsen, 1986) and is also known to exhibit great plasticity in of their life history patterns (Daverat et al., in press), thus residing in zones where the food availability and environmental conditions are optimal. In fact, this species can be spatially distributed in patches (Modin and Pihl, 1996).

The density of the European flounder juveniles in S1 (close to the river mouth) was the lowest found in the estuary, indicating that the Minho estuary population may prefer zones with lower values of salinity. Similar patterns of preference for low salinity were previously acknowledged in the literature (e.g. Kerstan, 1991; Van der Veer et al., 1991; Thiel et al., 2003; Martinho et al., 2007; Freitas et al., 2009).

Sexual segregation is a relatively common pattern in flatfish's distribution (see Becker, 1988); but this issue have be somehow neglected; one exception was Bartolino et al. (2011), which investigated the ontogenetic and sex-specific differences in habitat selection of the yellowfin sole *Limanda aspera* finding that sexual segregation is more evident in adult stages, with juveniles did not differing much in habitat preferences according to its sex (Bartolino et al., 2011), as recorded in Minho estuary for *P. flesus* juveniles. Interestingly, previous studies involving the *P. flesus* population patterns in Portuguese estuaries did not measured possible sexual segregation (e.g. Cabral et al., 2007; Martinho et al., 2007; Freitas et al., 2009).

Similarly with Freitas et al. (2009), higher densities of *P. flesus* were recorded during summer in Minho estuary; however, in the present study, high densities were also found during the spring. Differences were also recorded in the months where the density peaks between two consecutive years, indicating that abundance peaks may change over the

years according to changes in environmental conditions. Actually, interannual variations in fish abundance in estuaries are common, and can result from differences in recruitment success among years or periods (Potter et al., 2001; Maes et al., 2005). Furthermore, density peaks can also occur in different seasons, for instance in the Baltic Sea, the peak seem to occur in late summer and the autumn (see Aarnio, 2000).

High *P. flesus* densities were recorded in upstream stations during the autumn, while during the remaining seasons, density values in upstream stations decreased for less than 30% of the total, suggesting that perhaps juveniles may move from the polyhaline portion of the estuary into the tidal freshwater (TFW) area during autumn. The movement towards the TWF area of the estuary could be related with increased growth rates at intermediate salinities compared with extreme salinities (Gutt, 1985). In fact, Bos and Thiel (2006) found that 0-group flounder selected the lower salinity conditions in laboratory migration experiment. Nonetheless, the salinity found in S4 and S5 is always near 0.05, thus it should be expected that juveniles avoid to remain in an area with such low values of salinity, and other factors may be driven the upward movements of *P. flesus* juveniles during the autumn.

The ecological interaction among other co-occurring key-species, such as the common goby *Pomatoschistus microps*, the sand goby *P. minutus*, the shore crab *Carcinus maenas* and the Great cormorant *Phalacrocorax carbo* might also be related with the different patterns of abundance of *P. flesus* juveniles among sampling stations. During autumn, the mean density of the common goby *Pomatoschistus microps* (288.4 ind.100m⁻²), the sand goby *P. minutus* (5.2 ind.100m⁻²) and the shore crab *Carcinus maenas* (57.2 ind.100m⁻²) were high in the lower (S1 and S2) and in the intermediary (S3) estuary compared with the remaining seasons (Souza et al., unpublished results); with flounder presenting low values in these three sampling stations (1.6 ± 0.49

ind.100m⁻²); but reaching densities three fold higher (5.1 ± 1.10 ind.100m⁻²) in the upstream stations (S4 and S5). The high densities of these species may favor the upstream dislocation of juvenile flounder, since they can possibly be competing for space and/or food. In fact, Złoch and Sapota (2010) found a niche overlap of 40% between *P. flesus* and *P. microps* and *P. minutus*, suggesting that competition between these species can also be occurring in Minho estuary. Additionally, it is possible that *P. flesus* might be preyed by the Shore crab and the Great cormorant in the lower and the intermediary estuary. Inside estuaries, fishes represent an important fraction of the *C. maenas* diet (see Baeta et al., 2006), while *P. flesus* represent *c.a.* 30% of total number of preys consumed by the Great cormorant, being the most representative food item of the species in Minho estuary (Dias et al., 2012). Of the four abovementioned species, only the Great cormorant co-occurs with flounder in the upstream stations, but the occurrence of *P. carbo* near S4 and S5 is noticeably lower than in the lower estuary (personal observation), indicating that in the upstream stations, *P. flesus* may find less competition and predators, thus being a favorable location for juveniles, particularly during the autumn.

The seasonal differences in the density of the European flounder among sampling stations can also be related with changes in the abundance of its preys, since *P. flesus* can actively move towards areas where its preys are more abundant (Modin and Pihl, 1996). Nevertheless, due to the lack of published information regarding the spatio-temporal density of the subtidal macrozoobenthic assemblages in Minho estuary, we cannot confirm that the availability of *P. flesus* preys is greater during autumn in upstream areas of the estuary or not. Further studies involving *P. flesus* and subtidal macrozoobenthos density in Minho estuary are necessary in order to confirm this possible correlation.

Despite of the spatio-temporal differences in *P. flesus* density patterns found in this study, we cannot conclusively say that the European flounder can move from one estuarine area to another, since we did not use appropriated techniques to measure fish dislocations. Therefore, further studies are necessary to describe *P. flesus* movements within the estuary.

The absence of larger *P. flesus* in all studies conducted in Minho estuary so far (e.g. Freitas et al., 2009; Dolbeth et al., 2010; our study) can be related with the sampling strategy adopted in all of them (1m beam trawl tows), which is a selective sampling gear for smaller size *P. flesus*. On the other hand, studies that used a different sampling gear (2m beam trawl) found much lower density values of *P. flesus* in Minho estuary than the present study (e.g. Vasconcelos et al., 2010; França et al., 2011), indicating that Minho's population may be primarily constituted by juveniles indeed. Still, larger flounders were occasionally recorded in upper Minho estuary (c.a. 70km upstream) (C. Antunes unpublished results).

Recruitment occurred during winter (March) in 2009 and during the spring in 2010 (May). This result is in accordance with Dolbeth et al. (2010), which identified the recruitment of a single cohort of *P. flesus* per year (during spring) in Minho and Mondego estuaries between 2004 and 2007. Differences in the months when *P. flesus* recruitment occurs may perhaps be related with differences in environmental conditions between years (Philippart et al. 1996).

Flounder distribution in coastal and estuarine systems is strongly influenced by a number of abiotic and biotic factors (Able et al., 2005). For flatfishes many studies indicate depth, temperature, salinity and substratum type as the best environmental predictors of habitat use within a study area (Able et al., 2005). In our study we found that these abiotic features were also good predictors of *P. flesus* distribution. However,

fishes from different size classes, showed marked differences in habitat preference in Minho, indicating that fishes from different sizes might be spatially segregated. It is important to highlight that the smallest fish (class F1) showed a strong correlation with low values of salinity and high values of temperature, indicating that these fish preferred to inhabit areas with low salinities (< 1), namely S3 (during winter and spring), S4 and S5. On the other hand, larger juveniles, tended to be associated with higher values of salinity, temperature and depth, indicating that these fishes (classes F5 and F6) tended to remain in the lower (S1 and S2) and the intermediary (S3) estuary. Otherwise, the intermediate size classes (F2, F3 and F4) showed weaker association with the environmental variables measured in this study thus can be associated either with the downstream or upstream estuarine zones.

Salinity is known to greatly influence the distribution of *P. flesus*, in fact, within estuaries, densities of juvenile *P. flesus* significantly increased with decreasing salinity (Kerstan, 1991; Van der Veer et al., 1991; Thiel et al., 2003; Martinho et al., 2007; Freitas et al., 2009), suggesting that there is an active choice of juveniles for low salinity waters (Bos and Thiel, 2006). Our results, however, showed that different size classes of *P. flesus* have different association with salinity, emphasizing that even during the juvenile phase, preferences can be different.

4.2. Flounder condition and production

In allometric equations for fish length and weight relationship, high allometric coefficient b values may indicate that fish present good condition, and the results obtained in Minho estuary were higher than those recorded in other places (see Table 4). This could be an indicative that in Minho estuary, *P. flesus* may find optimal conditions for growth, thus helping to explain the high flounder density observed in this estuary

compared with other locations. Additionally, the second highest *b* value was also found in an estuarine system (Mirna estuary in Croatia) (Dulcic and Glamuzina, 2006). Inside estuaries, fishes find favorable conditions for rapid growth due to the higher availability of food and shelter (Haedrich, 1983), and not arbitrarily, these locations function as nursery grounds (Beck et al., 2001). On the other hand, the high fish condition found in this study might be related with the small range in fish size observed in Minho estuary compared with other systems (see Table 4). Fish condition can vary ontogenetically (Azuma et al., 1998), with smaller juveniles presenting higher condition than larger juveniles and subadults (Costa and Araújo, 2003). Therefore, the higher condition of *P. flesus* in Minho estuary could be related with the dominance of small-bodied individuals in the population.

The high fish condition measured for *P. flesus* in Minho estuary, associated with the noticeably high densities of several aquatic species, such as the Asian clam *C. fluminea* (Sousa et al., 2008c), the common goby *P. microps* (Souza et al., unpublished results), the sand goby *P. minutus* (Souza et al., unpublished results) the shore crab *C. maenas* (Souza et al., unpublished results) and also the European flounder *P. flesus* (Cabral et al., 2007; Freitas et al., 2009, our study) found in Minho estuary, can be an indicative that this estuary presents good condition for this and other aquatic species (Gibson, 1994; Amara et al., 2009). This fact may perhaps be related to the higher habitat preservation of Minho estuary compared to other estuaries (see Sousa et al., 2008d).

The secondary production of *P. flesus* was lower than previously reported for Minho (39 times lower) and Mondego (2.7 times lower) estuaries (see Dolbeth et al., 2010). Similarly with Dolbeth et al. (2010), we performed diurnal tows using the same sampling gear (1m beam trawl with 5 mm mesh size), but our sampling strategy was highly different, since we performed replicates in each of the five sampling stations

(from the first 29km of the estuary), while they used data only from 6 sampling stations located in the first 12km of the estuary with no replicates within each sampling station. In our study, most of the individuals captured were very small in size, and interestingly smaller fishes were sampled in the areas not sampled by Dolbeth et al. (2010). Also, the mean density value found in our study ($2.84 \pm 0.27 \text{ ind.}100\text{m}^{-2}$) was lower than previous studies conducted in Minho (see Freitas et al., 2009; Dolbeth et al., 2010), and since production is directly related with fish size and density, it should be expected that our production estimate would be lower than theirs' and most of the dissimilarities obtained by both studies might possibly be related to differences in the experimental design.

5. Conclusions

The juvenile flounder population in Minho estuary was spatially and temporally structured, with marked differences in habitat preferences among size classes. Flounders also seem to perform upstream dislocations during autumn, probably due to better environmental conditions for the species found in this portion of the estuary during this season compared with the lower and the intermediary estuary. Flounders from Minho estuary had higher densities (see Vinagre et al., 2005; Pombo et al., 2007; Martinho et al., 2007; Ramos et al., 2010; Vasconcelos et al., 2010; França et al., 2011) and condition (see Bedford et al., 1986; Dorel, 1986; Coull et al., 1989; Vianet et al., 1989; Froese and Freiß, 1992; Dulcic and Glamuzina, 2006) when compared with other areas, indicating that this estuary might present better conditions for the growth of *P. flesus* juveniles. Nonetheless, the secondary production obtained in our study was lower than previous studies acknowledged.

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6. References

- Aarnio, K., 2000. Experimental evidence of predation by juvenile flounder *Platichthys flesus*, on a shallow water meiobenthic community. J. Exp. Mar. Biol. Ecol. 246, 125–138.
- Able, K.W., 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuar. Coast. Shelf. Sci. 64, 5–17.
- Able, K.W., Neuman, M.J., Wennhage, H., 2005. Ecology of juvenile and adult stages of flatfishes: distribution and dynamics of habitat associations, in: Gibson R.N., (Ed.), Flatfishes: Biology and Exploitation. Blackwell Science, Oxford, pp. 164–184.
- Alves, A.M., 1996. Causas e processos da dinâmica sedimentar na evolução actual do litoral do Alto Minho. PhD Thesis, Universidade do Minho, Portugal.

- Amara, R., Selleslagh, J., Billon, G., Minier, C., 2009. Growth and condition of 0-group European flounder, *Platichthys flesus* as indicator of estuarine habitat quality. *Hydrobiologia* 627, 87–98.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26, 32–46.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth.
- Antunes, C., Araújo, M.J., Braga, C., Roleira, A., Carvalho, R., Mota, M., 2011. Valorização dos recursos naturais da bacia hidrográfica do rio Minho. Final report from the project Natura Miño-Minho, Centro interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto.
- Azuma, T., Yada, T., Ueno, Y., Iwata, M., 1998. Biochemical approach to assessing growth characteristics in salmonids. *N. Pac. Anadr. Fish. Comm. Bull.* 1, 103–111.
- Baeta, A., Cabral, H.N., Marques, J.C., Pardal, M.A., 2006. Feeding ecology of the green crab, *Carcinus maenas* (L., 1758) in a temperate estuary, Portugal. *Crustaceana*. 79(10), 1181–1193.
- Bain, M.B., Finn, J.T., Booke, H.E., 1988. Streamflow regulation and fish community structure. *Ecology*. 69, 182–192.
- Bartolino, V., Ciannelli, L., Bacheler, N.M., Chan, K.-S., 2011. Ontogenetic and sex-specific differences in density-dependent habitat selection of a marine fish. *Ecology*. 92(1), 189–200.
- Beck, M.V., Kennen, L., Heck, J.R., Kennen, W.A., Childers, D.L., Eggleston D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The identification, conservation, and

- management of estuarine and marine nurseries for fish and invertebrates. Bioscience. 51, 633–641.
- Becker, D.S., 1988. Relationship between sediment character and sex segregation in English sole, *Parophrys vetulus*. Fish. B-NOAA. 86(3), 517–524.
- Bedford, B.C., Woolner, L.E., Jones, B.W., 1986. Length-weight relationships for commercial fish species and conversion factors for various presentations. Ministry of Agriculture, Fisheries and Food. Directorate of Fisheries Research. Fisheries Research Data Report No. 10.
- Beverton, R.J.H., Holt, S.J., 1996. On the dynamics of exploited fish populations. Chapman and Hall, London, 533pp.
- Bos, A.R., Thiel, R., 2006. Influence of salinity on the migration of postlarval and juvenile flounder *Pleuronectes flesus* (L.) in gradient experiment. J. Fish Biol. 68, 1411–1420.
- Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P., Costa, M.J., 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. J. Sea Res. 57, 209–217.
- Chambers, J.R., 1992. Coastal degradation and fish population losses, in: Stroud R.S. (Ed.), Stemming the tide of coastal fish habitat loss. National Coalition for Marine Conservation, Inc., Leesburg, pp. 45–51.
- Chesson, P., 1998. Making sense of spatial models in ecology. Modelling spatiotemporal dynamics in ecology, in: Bascompte, J., Sole, R.V., (Eds.). Springer-Verlag, Berlin, pp. 151–166.
- Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User manual/tutorial. PRIMER-E, Plymouth.

- Costa, M.R., Araújo, F.G., 2003. Length-weight relationship and condition factor of *Micropogonias furnieri* (Desmarest) (Perciformes, Sciaenidae) in Sepetiba Bay, Rio de Janeiro State, Brazil. *Rev. Bras. Zool.* 20(4), 685–690.
- Coull, K.A., Jermyn, A.S., Newton, A.W., Henderson, G.I., Hall, W.B., 1989. Length/weight relationships for 88 species of fish encountered in the North Atlantic. *Scottish Fish. Res. Rep.* 43.
- Daverat, F., Morais, P., Dias, E., Martin, J., Babaluk, J., Fablet, R., Peycheran, C., Antunes, C., in press. Plasticity of European flounder life history patterns discloses alternatives to catadromy. *Mar. Ecol. Progr. Ser.*
- Dempster, A.P., Laird, N.M., Rubin, D.B., 1977. Maximum likelihood from incomplete data via EM algorithm. *J. Roy. Stat. Soc. B.* 39, 1–38.
- Dias, E., Morais, P., Leopold, M., Campos, J., Antunes, C., in press. Natural born indicators: great cormorant *Phalacrocorax carbo* (Aves: Phalacrocoracidae) as monitors of river discharge influence on estuarine ichthyofauna. *J. Sea. Res.* 73, 101–108.
- Dolbeth, M., Martinho, F., Viegas, I., Cabral, H., Pardal, M.A., 2008. Estuarine production of resident and nursery fish species: conditioning by drought events? *Estuar. Coast. Shelf. Sci.* 78, 51–60.
- Dolbeth, M., Martinho, F., Freitas, V., Costa-Dias, S., Campos, J., Pardal, M.A., 2010. Multi-year comparisons of fish recruitment, growth and production in two drought-affected Iberian estuaries. *Mar. Freshw. Res.* 61, 1399–1415.
- Dorel, D., 1986. Poissons de l’Atlantique nord-est relations taille-poids. Institut Français de Recherche pour l’Exploitation de la Mer. Nantes.

- Dulcic, J., Glamuzina, B., 2006. Length-weight relationships for selected fish species from three eastern Adriatic estuarine systems (Croatia). *J. Appl. Ichthyol.* 22, 254–256.
- Ferreira, J.G., Simas, T., Nobre, A., Silva, M.C., Schifferegger, K., Lencart-Silva, J., 2003. Identification of sensitive areas and vulnerable zones in transitional and coastal Portuguese systems. Application of the United States National Estuarine Eutrophication Assessment to the Minho, Lima, Douro, Ria de Aveiro, Mondego, Tagus, Sado, Mira, Ria Formosa and Guadiana systems. INAG/IMAR Technical Report.
- França, S., Costa, M. J., Cabral, H. N., 2011. Inter- and intra-estuarine fish assemblage variability patterns along the Portuguese coast. *Estuar. Coast. Shelf. Sci.* 91, 262–271.
- Freitas, V., Costa-Dias, S., Campos, J., Bio, A., Santos, P., Antunes, C., 2009. Patterns in abundance and distribution of the juvenile flounder, *Platichthys flesus*, in Minho estuary (NW Iberian Peninsula). *Aquat. Ecol.* 43, 1143–1153.
- Froese, R., Freiß, C.C., 1992. Synopsis of biological data on *Platichthys flesus* (L.), ICES assessment units 22 and 24, using the FISHBASE format. ICES C.M.1992/J:41. International Council for the Exploration of the Sea, Copenhagen.
- Froese, R., Pauly, D., 2012. Fishbase. <http://www.fishbase.org>. Version February 2012.
- Garrison, L., Link, J.S., 2000. Diets of five hake species in the northeast United States continental shelf ecosystem. *Mar. Ecol. Progr. Ser.* 204, 243–255.
- Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands J. Sea Res.* 32, 191–206.
- Gorman, O.T., Karr, J.R., 1978. Habitat structure and stream fish communities. *Ecology.* 59, 507–515.

- Gutt, J., 1985. The growth of juvenile flounders (*Platichthys flesus* L.) at salinities of 0, 5, 15 and 35‰. J. Appl. Ichthyol. 1, 17–26.
- Haedrich, R.L., 1983. Estuarine fishes, in: Ketchum B.H. (Ed.), Estuaries and Enclosed Seas. Elsevier Publishing Company, Amsterdam, pp. 183–207.
- Hoffman, J.C., Bronk, D.A., Olney, J.E., 2008. Organic matter sources supporting lower food web production in the tidal freshwater portion of the York River estuary, Virginia. Estuar. Coast. 31, 898–911.
- Ilari, M.I., Freitas, F., Costa-Dias, S., Antunes, C., Guilhermino, L., Sousa, R., 2012. Associated macrozoobenthos with the invasive Asian clam *Corbicula fluminea*. J. Sea. Res. 72, 113–120.
- Kerstan, M., 1991. The importance of rivers as nursery grounds for 0- and 1-group flounder (*Platichthys flesus* L.) in comparison to the Wadden sea. Neth. J. Sea. Res. 27, 353–366.
- Kosior, M., Kuczynski, J., Grygiel, W., 1996. Reproduction of Baltic flounder (*Platichthys flesus* (L.)) in relation to some somatic factors. ICES Counc. Meet. Pap., 25pp.
- Křivan, V., Siroť, E., 2002. Habitat selection by two competing species in a two-habitat environment. Am. Nat. 160, 214–234.
- Krueger, C.C., Martin, F.B., 1980. Computation of confidence intervals for size-frequency (Hynes) method of estimating secondary production. Limnol. Oceanogr. 25(4), 773–777.
- Maes, J., Stevens, M., Ollevier, F., 2005. The composition and community structure of the ichthyofauna of the upper Scheldt estuary: synthesis of a 10-year data collection (1991-2001). J. Appl. Ichthyol. 21, 86–93.

- Martinho, F., Leitão, R., Neto, J.M., Cabral, H.N. Marques, J.C., Pardal, M.A., 2007. The use of nursery areas by juvenile fish in a temperate estuary, Portugal. *Hydrobiologia*. 587, 281–290.
- Martinho, F., Dolbeth, M., Viegas, I., Teixeira, C.M., Cabral, H.N., Pardal, M.A., 2009. Environmental effects on the recruitment variability of nursery species. *Estuar. Coast. Shelf. Sci.* 83, 460–468.
- Matthews, W.J., 1998. Patterns in freshwater fish ecology. Chapman & Hall, London.
- Mérigoux, S., Ponton, D., de Mérona, B., 1998. Fish richness and species-habitat relationships in two coastal streams in French Guyana, South America. *Environ. Biol. Fish.* 51, 25–39.
- Modin, J., Pihl, L., 1996. Small-scale distribution of juvenile plaice and flounder in relation to predatory shrimp in a shallow Swedish bay. *J. Fish. Biol.* 49(6), 1070–1085.
- Morais, P., Dias, E., Babaluk, J., Antunes, C. 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: Ecological implications and fishery management. *J. Sea Res.* 65, 235–246.
- Munroe, T.A., 2010. *Platichthys flesus*. In: IUCN 2011. IUCN Red List of Threatened Species. <http://www.iucnredlist.org>. Version 2011.2.
- Nelson, D.M., Monaco, M.E., 2000. National Overview and Evolution of NOAA's Estuarine Living Marine Resources (ELMR) Program. NOAA Tech. Memo. NOS NCCOS CCMA 144. Silver Spring, MD: NOAA, NOS, Center for Coastal Monitoring and Assessment.

- Nielsen, J.G., 1986. Pleuronectidae, in: Whitehead, P.J.P., Bauchot, M.-L., Hureau, J.-C., Nielsen J., Tortonese E. (Eds.), Fishes of the North-eastern Atlantic and the Mediterranean. UNESCO, Paris. pp. 1299–1307.
- Philippart, C.J.M., Lindeboom, H.J., van der Meer, J., van der Veer, H.W., Witte, J.I.J., 1996. Long-term fluctuations in fish recruit abundance in the western Wadden Sea in relation to variation in the marine environment. ICES J. Mar. Sci. 53, 1120–1129.
- Pombo, L., Rebelo, J.E., Elliott, M., 2007. The structure diversity and somatic production of the fish community in an estuarine lagoon, Ria de Aveiro (Portugal). Hydrobiologia. 587, 253–268.
- Potter, I.C., Bird, D.J., Claridge, P.N., Clarke, K.R., Hyndes, G.A., Newton, L.C., 2001. Fish fauna of the Severn Estuary. Are there long-term changes in abundance and species composition and are the recruitment patterns of the main marine species correlated? J. Exp. Mar. Biol. Ecol. 258, 15–37.
- Ramos, S., Ré, P., Bordalo, A.A., 2009. Environmental control on early life stages of flatfishes in Lima Estuary (NW Portugal). Estuar. Coast. Shelf. Sci. 83, 252–264.
- Ramos, S., Ré, P., Bordalo A.A., 2010. Recruitment of flatfish species to an estuarine nursery habitat (Lima estuary, NW Iberian Peninsula). J. Sea Res. 64, 473–486.
- Reiss, M.J., 1989. The allometry of growth and reproduction. Cambridge University Press, Cambridge.
- Rochard, E., Elie, P., 1994. La macrofaune aquatique de l'estuaire de la Gironde. Contribution au livre blanc de l'Agence de l'Eau Adour Garonne, in: Mauvais J.-L., Guillaud J.-F. (Eds.) État des connaissances sur l'estuaire de la Gironde. Agence de l'Eau Adour-Garonne, Éditions Bergeret, Bordeaux, France. pp. 1–56.

- Schlosser, I.J., 1982. Fish community structure and function along two habitat gradients in a headwater stream. *Ecol. Monogr.* 52, 395–414.
- Sousa, R., Dias, S., Freitas, V., Antunes, C., 2008a. Subtidal macrozoobenthic assemblages along the River Minho estuarine gradient (north-west Iberian Peninsula). *Aquat. Conserv. Mar. Freshw. Ecosyst.* 18, 1063–1077.
- Sousa, R., Rufino, M., Gaspar, M., Antunes, C., Guilhermino, L., 2008b. Abiotic impacts on spatial and temporal distribution of *Corbicula fluminea* (Müller, 1774) in the River Minho estuary, Portugal. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 18, 98–110.
- Sousa, R., Nogueira, A.J.A., Gaspar, M., Antunes, C., Guilhermino, L., 2008c. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): possible implications for ecosystem functioning. *Estuar. Coast. Shelf. Sci.* 80, 289–295.
- Sousa, R., Costa-Dias, S., Guilhermino, L., Antunes, C., 2008d. Minho River tidal freshwater wetlands: threats to faunal biodiversity. *Aquat. Biol.* 3, 237–250.
- Souza, A.T., Ilarri, M.I., Campos, J., Marques J.C., Martins, I., 2011. Differences in the neighborhood: Structural variations in the carapace of shore crabs *Carcinus maenas* (Decapoda: Portunidae). *Estuar. Coast. Shelf. Sci.* 95, 424–430.
- Swain, D., Chouinard, G.A., Morin, R., Drinkwater, K.F., 1998. Seasonal variation in the habitat associations of Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*) from the southern Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* 55, 2548–2561.
- ter Braak, C.J.F., 1986. Canonical Correspondence Analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology.* 67, 1167–1179.

- ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquat. Sci.* 57, 255–289.
- Thiel, R., Cabral, H., Costa, M.J., 2003. Composition, temporal changes and ecological guild classification of the ichthyofaunas of large European estuaries – a comparison between Tagus (Portugal) and Elbe (Germany). *J. Appl. Ichthyol.* 19, 330–342.
- Van der Veer, H.W., Bergman, M.J.N., Dapper, R., Witte, J.I.J., 1991. Population dynamics of an intertidal 0-group flounder *Platichthys flesus* population in the western Dutch Wadden Sea. *Mar. Ecol. Progr. Ser.* 73, 141–148.
- Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Maia, A., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuar. Coast. Shelf. Sci.* 79, 317–327.
- Vasconcelos, R. P., Reis-Santos, P., Maia, A., Fonseca, V., França, S., Wouters, N., Costa, M. J., Cabral, H. N., 2010. Nursery use patterns of commercially importante marine fish species in estuarine systems along the Portuguese coast. *Estuar. Coast. Shelf. Sci.* 86, 613–624.
- Vianet, R., Quignard, J.-P., Tomasini, J.-A., 1989. Age et croissance de quatre poissons Pleuronectiformes (flet, turbot, barbue, sole) du golfe du Lion. *Cybiuim.* 13(3), 247–258.
- Vinagre C., França, S., Costa, M.J., Cabral, H.N. 2005. Niche overlap between juvenile flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjacent coastal waters. *J. Appl. Ichthyol.* 21, 114–120.
- Złoch, I., Sapota, M.R., 2010. Trophic interactions between preadult and adult *Pomatoschistus minutus* and *Pomatoschistus microps* and young *Platichthys*

flesus occurring in inshore waters of the Gulf of Gdansk (Southern Baltic).

Oceanol. Hydrobiol. St. 39(2), 37–53.

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Figure legends

Fig. 1 Map of the study site showing the five sampling stations in Minho estuary, NW Iberian Peninsula.

Fig. 2 Principal Components Analysis (PCA) biplot for the abiotic data collected from February 2009 to July 2010 in five sampling stations (S1, S2, S3, S4 and S5) in Minho estuary, NW Iberian Peninsula.

Fig. 3 (A) Mean density (\pm SE) of the European flounder *Platichthys flesus* during 18-months study period in Minho estuary. (B) Comparisons on the densities (mean \pm SE) of *P. flesus* between sampling stations and seasons. Different letters (a, b, c or d) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colors (black or white), while continuous line indicates that there are no significant differences among seasons crossed by it (Two-way PERMANOVA pairwise tests: $p < 0.05$).

Fig. 4 (A) Size classes proportions of the European flounder *Platichthys flesus* in Minho estuary throughout time. Size classes: F1 (< 25 mm), F2 (25 to 49.9 mm), F3 (50 to 74.9 mm), F4 (75 to 99.9 mm), F5 (100 to 124.9 mm) and F6 (> 125 mm). Vertical dashed lines separate different seasons. (B) Mean cohort length of *P. flesus* in Minho estuary, with indication of the three cohorts identified by the mixture analysis (C1, C2 and C3).

Fig. 5 Canonical plotting of environmental characteristics (arrows) and different size classes of the European flounder *Platichthys flesus* in Minho estuary, NW Iberian Peninsula. ORP = Redox potential. Size classes: F1 (< 25 mm), F2 (25 to 49.9 mm), F3 (50 to 74.9 mm), F4 (75 to 99.9 mm), F5 (100 to 124.9 mm) and F6 (> 125 mm).

Table legends

Table 1 Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term on the abiotic variables in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

Table 2. Comparisons on the density (mean \pm SE) of males and females of the European flounder *Platichthys flesus* among five sampling stations and four seasons in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

Table 3 Comparisons on the standard length (mean \pm SE) of males and females of the European flounder *Platichthys flesus* among sampling stations and seasons in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.01$ *** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

Table 4 Comparison of the values obtained by length and weight relationship of the European flounder *Platichthys flesus* in different locations.

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Figures

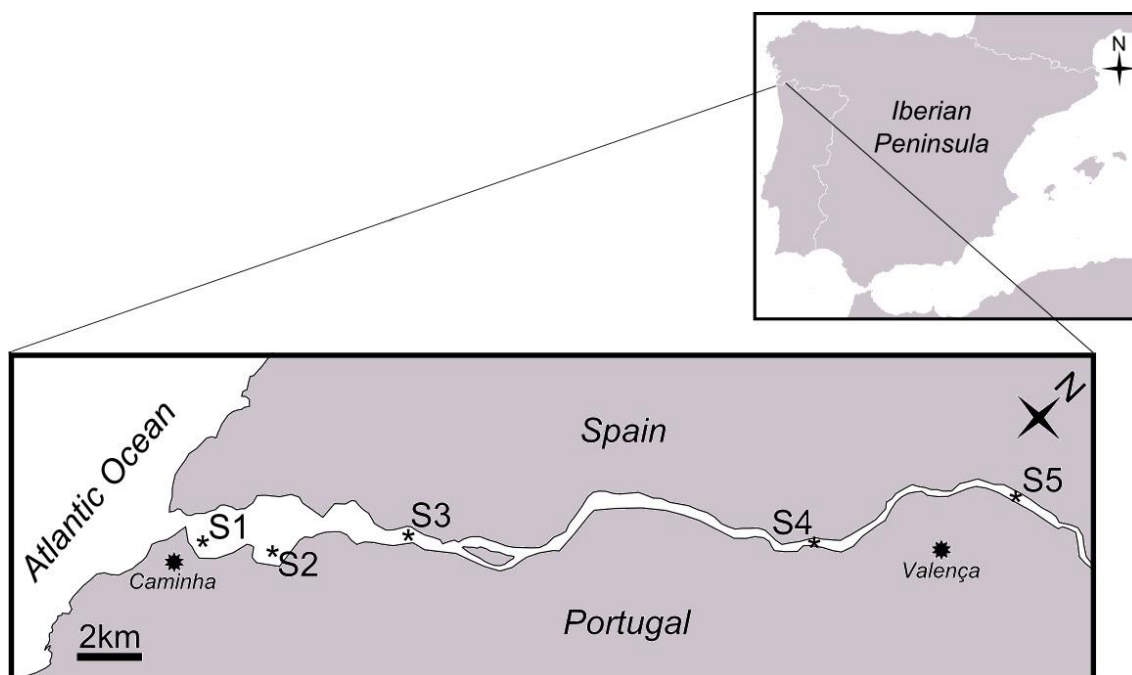


Fig. 1

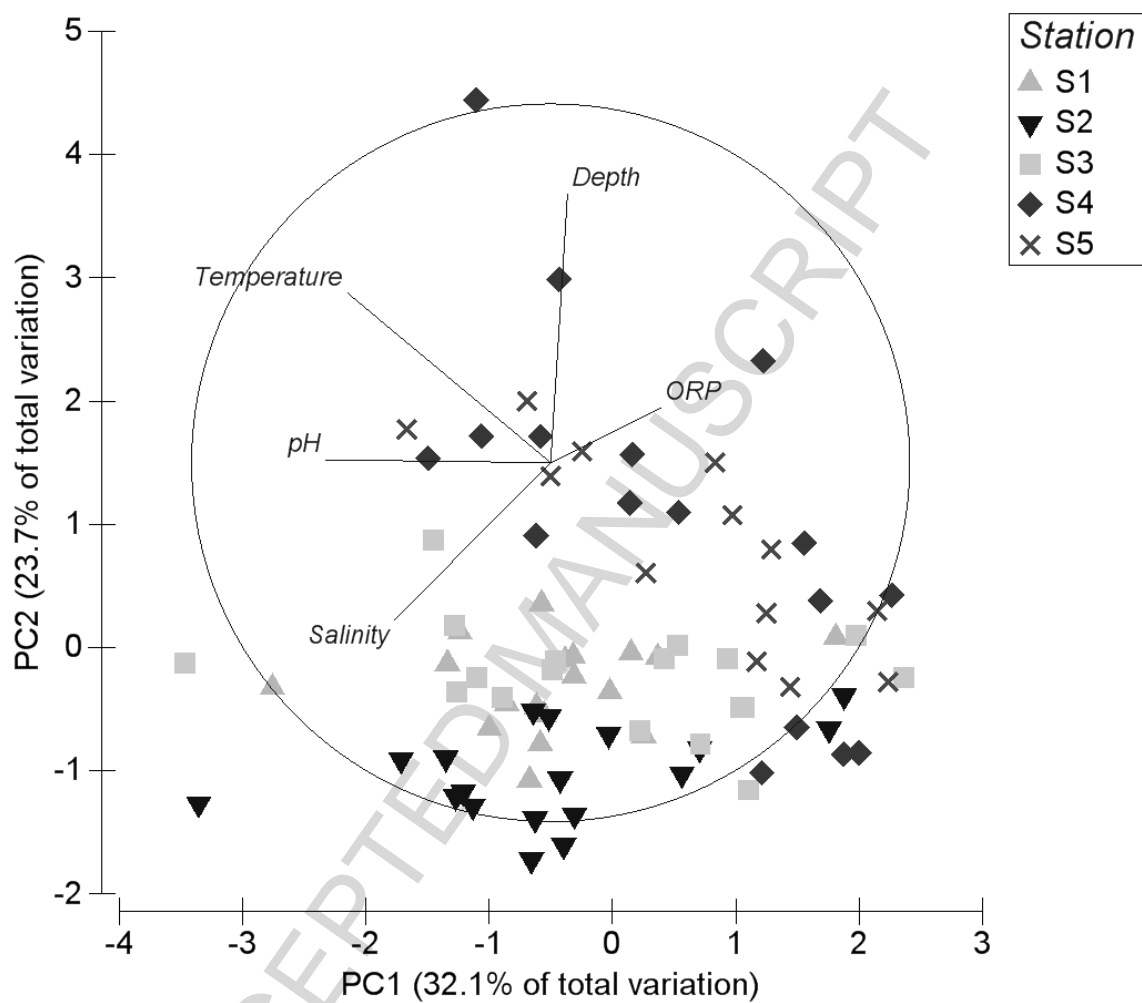


Fig. 2

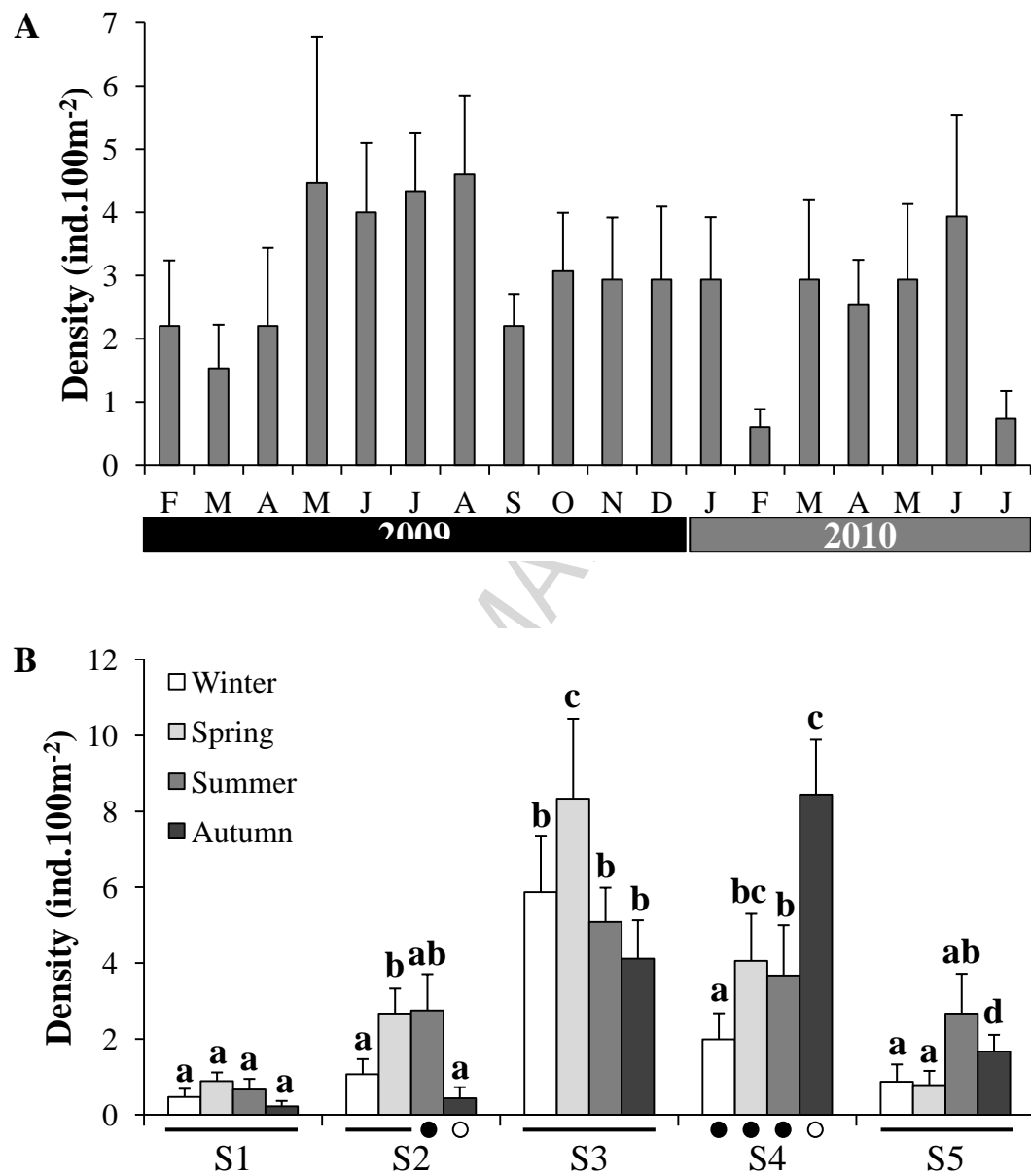


Fig. 3

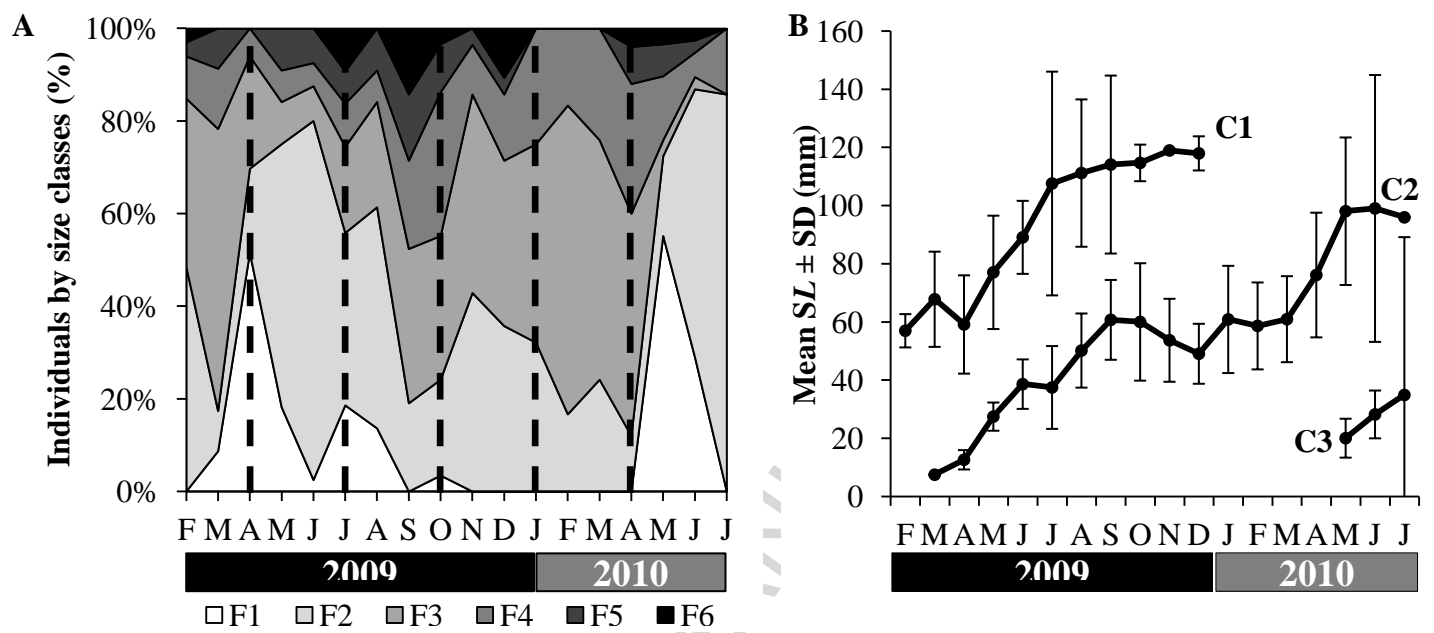


Fig. 4

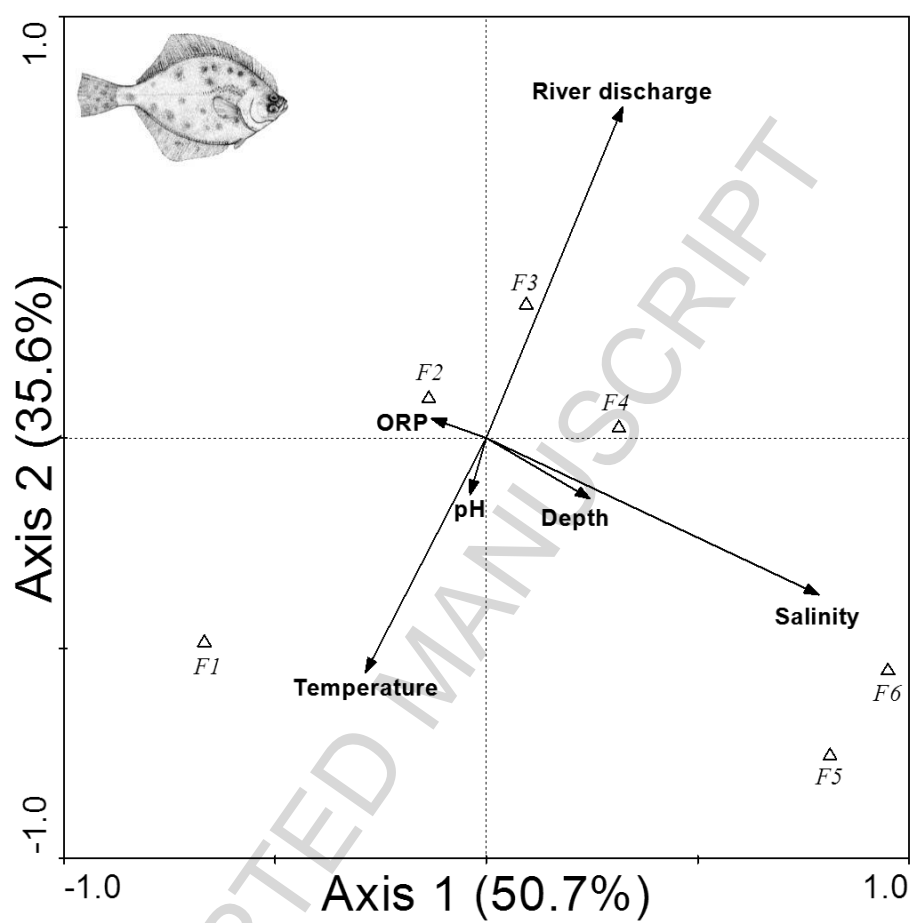


Fig. 5

Tables

Table 1

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Depth							
S1	$4.17 \pm 0.29^{ad(A)}$	$3.08 \pm 0.31^{b(A)}$	$3.69 \pm 0.16^{bd(A)}$	$4.33 \pm 0.08^{ac(A)}$			
S2	$1.90 \pm 0.24^{(B)}$	$1.28 \pm 0.19^{(B)}$	$1.84 \pm 0.09^{(B)}$	$1.83 \pm 0.12^{(B)}$	Pseudo- <i>F</i> = 22.20***	Pseudo- <i>F</i> = 3.81*	Pseudo- <i>F</i> = 2.53**
S3	$2.74 \pm 0.43^{(BC)}$	$1.89 \pm 0.28^{(B)}$	$2.75 \pm 0.27^{(C)}$	$2.05 \pm 0.10^{(B)}$			
S4	$2.36 \pm 0.43^{a(BC)}$	$4.29 \pm 0.64^{ab(AC)}$	$5.88 \pm 1.20^{b(A)}$	$3.90 \pm 0.40^{ab(A)}$			
S5	$4.30 \pm 0.40^{(AC)}$	$3.30 \pm 0.55^{(AC)}$	$4.27 \pm 0.15^{(A)}$	$3.97 \pm 0.64^{(A)}$			
ORP							
S1	180.80 ± 37.60	156.10 ± 28.90	144.40 ± 36.30	169.20 ± 27.00	Pseudo- <i>F</i> = 0.68 ^{ns}	Pseudo- <i>F</i> = 0.95 ^{ns}	Pseudo- <i>F</i> = 0.21 ^{ns}
S2	189.10 ± 45.60	153.60 ± 32.0	159.50 ± 41.50	173.40 ± 19.00			
S3	201.20 ± 52.20	159.90 ± 31.50	167.20 ± 43.70	170.60 ± 26.70			

S4	235.80 ± 59.60	190.30 ± 41.20	165.70 ± 23.70	189.40 ± 21.90			
S5	264.10 ± 46.70	247.00 ± 0.50	157.00 ± 18.20	206.20 ± 38.80			
pH							
S1	7.83 ± 0.24	7.83 ± 0.27	8.54 ± 0.27	7.96 ± 0.23			
S2	7.77 ± 0.16 ^a	8.02 ± 0.21 ^{ab}	8.81 ± 0.32 ^b	8.05 ± 0.28 ^{ab}	Pseudo- <i>F</i> =	Pseudo- <i>F</i> =	Pseudo- <i>F</i> =
S3	7.69 ± 0.12 ^a	8.05 ± 0.21 ^{ab}	8.88 ± 0.34 ^b	8.13 ± 0.22 ^a	1.99 ^{ns}	8.75***	0.35 ^{ns}
S4	7.57 ± 0.19	7.69 ± 0.26	8.08 ± 0.28	7.96 ± 0.41			
S5	7.75 ± 0.09	7.70 ± 0.05	8.10 ± 0.39	7.88 ± 0.32			
Salinity							
S1	25.75 ± 6.49 ^(A)	25.98 ± 2.94 ^(A)	32.00 ± 0.96 ^(A)	31.78 ± 1.91 ^(A)			
S2	12.77 ± 7.76 ^(AB)	15.85 ± 6.23 ^(AD)	30.79 ± 1.86 ^(A)	23.03 ± 6.86 ^(AB)	Pseudo- <i>F</i> =	Pseudo- <i>F</i> =	Pseudo- <i>F</i> =
S3	0.38 ± 0.22 ^{a(B)}	0.36 ± 0.24 ^{a(BC)}	19.06 ± 4.24 ^{b(B)}	4.26 ± 4.19 ^{ab(BC)}	41.47***	4.85**	1.21 ^{ns}
S4	0.04 ± 0.00 ^(B)	0.05 ± 0.01 ^(BC)	0.05 ± 0.00 ^(C)	0.04 ± 0.01 ^(C)			
S5	0.05 ± 0.01 ^(B)	0.05 ± 0.01 ^(BCD)	0.05 ± 0.00 ^(C)	0.05 ± 0.01 ^(C)			
Temperature							

S1	11.88 ± 0.86^a	14.58 ± 0.51^b	$14.99 \pm 0.57^{b(A)}$	15.82 ± 0.43^b	Pseudo- <i>F</i> = 1.12 ^{ns}	Pseudo- <i>F</i> = 48.50***	Pseudo- <i>F</i> = 3.29***
S2	11.69 ± 0.76^a	16.37 ± 0.85^b	$15.90 \pm 0.48^{b(AB)}$	15.10 ± 1.45^{ab}			
S3	9.56 ± 0.78^a	16.44 ± 1.19^b	$18.32 \pm 1.16^{b(B)}$	14.68 ± 1.82^b			
S4	9.77 ± 0.50^a	15.62 ± 1.44^b	$22.54 \pm 0.28^{c(C)}$	15.10 ± 2.24^b			
S5	9.90 ± 1.10^a	15.60 ± 2.15^b	$21.93 \pm 0.09^{c(C)}$	14.90 ± 2.15^b			

Table 2

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
S1	$0.20 \pm 0.20^{(A)}$	$0.44 \pm 0.17^{(A)}$	$0.50 \pm 0.29^{(A)}$	$0.22 \pm 0.15^{(A)}$			
S2	$0.53 \pm 0.21^{(A)}$	$1.22 \pm 0.37^{(A)}$	$1.00 \pm 0.48^{(A)}$	$0.22 \pm 0.22^{(A)}$			
S3	$2.87 \pm 0.79^{(B)}$	$4.61 \pm 1.21^{(B)}$	$3.17 \pm 0.63^{(B)}$	$2.33 \pm 0.60^{(B)}$	Pseudo- <i>F</i> = 16.43**	Pseudo- <i>F</i> = 1.04 ^{ns}	Pseudo- <i>F</i> = 2.44**
S4	$0.87 \pm 0.43^{a(A)}$	$1.44 \pm 0.60^{a(A)}$	$1.58 \pm 0.69^{a(AB)}$	$4.33 \pm 1.13^{b(B)}$			
S5	$0.47 \pm 0.24^{a(A)}$	$0.00 \pm 0.00^{b(C)}$	$1.08 \pm 0.50^{a(A)}$	$0.22 \pm 0.22^{ab(A)}$			
Female							

S1	$0.27 \pm 0.12^{(A)}$	$0.44 \pm 0.17^{(A)}$	$0.17 \pm 0.11^{(A)}$	$0.00 \pm 0.00^{(A)}$			
S2	$0.60 \pm 0.25^{(A)}$	$1.50 \pm 0.41^{(BC)}$	$1.67 \pm 0.64^{(B)}$	$0.22 \pm 0.22^{(A)}$			
S3	$3.07 \pm 0.87^{(B)}$	$3.39 \pm 0.98^{(C)}$	$2.25 \pm 0.54^{(B)}$	$1.89 \pm 0.70^{(BC)}$	Pseudo- <i>F</i>	Pseudo- <i>F</i>	Pseudo- <i>F</i>
					= 11.20**	= 0.93 ^{ns}	= 1.87*
S4	$0.87 \pm 0.32^{a(A)}$	$1.67 \pm 0.40^{a(BC)}$	$1.83 \pm 0.63^{a(B)}$	$3.89 \pm 0.68^{b(B)}$			
S5	$0.33 \pm 0.23^{a(A)}$	$0.78 \pm 0.38^{ab(AB)}$	$1.58 \pm 0.70^{ab(AB)}$	$1.44 \pm 0.47^{b(C)}$			

Table 3

	Standard length (mm)				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Station	Season	Interaction
Male							
S1	92.00 ± 11.60 ^(A)	112.44 ± 6.49 ^(A)	133.30 ± 16.10 ^(A)	168.50 ± 59.05 ^(A)	Pseudo- <i>F</i> = 48.67***	Pseudo- <i>F</i> = 7.90***	Pseudo- <i>F</i> = 3.39**
S2	65.70 ± 5.06 ^{a(B)}	63.96 ± 7.87 ^{a(C)}	62.90 ± 12.20 ^{a(B)}	208.50 ± 0.00 ^{b(A)}			
S3	63.44 ± 3.50 ^{a(B)}	38.20 ± 2.87 ^{b(B)}	55.37 ± 7.29 ^{a(B)}	65.13 ± 6.35 ^{a(B)}			
S4	47.83 ± 3.36 ^{a(C)}	34.97 ± 3.69 ^{b(B)}	39.50 ± 11.00 ^{ab(B)}	53.63 ± 4.25 ^{a(B)}			
S5	66.00 ± 10.60 ^(ABC)	-	66.60 ± 13.70 ^(B)	89.50 ± 0.00 ^(AB)			
Female							
S1	76.63 ± 8.10 ^(A)	107.60 ± 11.50 ^(A)	102.00 ± 14.00 ^(A)	-	Pseudo- <i>F</i> = 4.48*	Pseudo- <i>F</i> = 0.36 ^{ns}	Pseudo- <i>F</i> = 3.54**
S2	64.83 ± 6.23 ^(AB)	50.38 ± 5.05 ^(B)	63.33 ± 5.72 ^(B)	81.00 ± 0.00 ^(A)			
S3	60.00 ± 2.53 ^{a(B)}	39.24 ± 3.08 ^{b(B)}	60.81 ± 6.77 ^{a(AB)}	76.80 ± 15.00 ^{a(A)}			
S4	51.95 ± 5.32 ^{ab(B)}	52.08 ± 5.23 ^{ab(B)}	39.14 ± 3.75 ^{a(C)}	49.64 ± 2.61 ^{b(B)}			

S5	$47.38 \pm 3.88^{a(B)}$	$54.56 \pm 9.47^{a(B)}$	$56.50 \pm 6.73^{a(B)}$	$91.56 \pm 7.48^{b(A)}$
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Table 4

Country	Locality	allometric coefficient a	allometric coefficient b	Sex	TL range (cm)	Reference
Germany	Western Baltic	0.0220	2.830	Male	15.0 to 42.0	Froese and Freiß, 1992
Germany	Western Baltic	0.0158	2.956	Female	15.0 to 42.0	Froese and Freiß, 1992
France	East and West Channel	0.0116	2.963	Unsexed	5.0 to 48.0	Dorel, 1986
England	-	0.0125	2.968	Both	-	Bedford et al., 1986
France	Bay of Biscay	0.0093	3.066	Unsexed	-	Dorel, 1986
Scotland	Moray Firth and Buchan	0.0087	3.098	Unsexed	12.0 to 38.0	Coull et al., 1989
France	Gulf of Lyon	0.0063	3.100	Both	5.0 to 43.0	Vianet et al., 1989
Croatia	Mirna estuary	0.0070	3.110	Unsexed	11.0 to 43.0	Dulcic and Glamuzina, 2006
Portugal	Minho estuary	0.0045	3.310	Both	1.3 to 27.5	Present study
Portugal	Minho estuary	0.0048	3.267	Male	1.3 to 27.5	Present study

Portugal	Minho estuary	0.0041	3.363	Female	1.7 to 22.5	Present study
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Highlights

- Flounder densities in Minho estuary were up to 32 times higher than elsewhere
- Population of juvenile flounder in Minho is highly dense but low productive
- Flounder juveniles showed distinct habitat preferences according to their size